A CO_2 EVOLUTION STUDY

IN ITASCA STATE PARK, MINNESOTA

A PLAN B PAPER

RESEARCH PROBLEMS IN SILVICULTURE (FBio. 8100)

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

MAY 1975

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INTRODUCTION

This paper reports original research on litter decomposition in an aspen stand (*Populus tremuloides* Michx. and *P. grandidentata* Michx.) following overstory removal. The objectives were: 1) to determine the effect of exposure on the rate of litter decay, and 2) to determine to what extent changes in temperature and moisture explain differences in the rate of decay. Litter decomposition rates were based on evolution of CO_2 from the litter. Many attempts have been made by other workers to quantify the rate of CO_2 evolution from forest floors, but regardless of sophisticated instrumentation the results are questionable. The difficulty is due to high variance, arising from measurement error and natural variation. Similar problems were encountered in the work reported here and are given considerable attention.

LITERATURE REVIEW

The literature review is written with three objectives. The first is to give an overall view of the relationship between litter decomposition and CO_2 evolution. The second is to show the wide range of approaches used for measurement. The third is to evaluate sources of error.

Litter Decomposition and CO_2 Evolution

The amount of CO_2 evolving from a soil has been used as an index for many biological phenomena, such as microorganism activity, number of organisms, soil fertility, and amount of organic matter available for decomposition (Waksman and Starkey 1924). The most common uses are for numbers and activity of microorganism (Gray and Wallace 1957, Nelson and Sollins 1973, Reiners 1964, Witkamp 1971).

Both the physical and nutritional value of the litter affect decomposition rate. The pH, protein content, mineral content, lignin content, and carbon-nitrogen ratio interact to determine the major types of decomposers present. In hardwood litter, a high pH, protein level, and mineral content, with low lignin content and carbon-nitrogen ratio all favor bacterial decomposition (Witkamp 1963). In conifers low pH favors fungal decomposition. A study conducted by Ivarson and Sowden (1959) illustrates the effect of the kind of litter upon the types of decomposers present. The study showed that CO_2 evolution from coniferous litter treated with a fungicide is less than that from the same kind of litter treated with an anti-bacterial agent. But bacterial decomposition of hardwood litter proceeds at a higher rate than does fungal decomposition of conifer litter (Ivarson and Sowden 1959). Other workers found there is not a significant difference between rates of CO_2 evolution of different litter types within the same geographic location (Melin 1930, Reiners 1964, Witkamp 1966).

This inconsistency in the literature could be the result of factors other than simply species differences, such as samples coming from different geographic regions. This results in organisms which differ in temperature and moisture optima. Another possible explanation is that variance in

quantifying CO_2 evolved is usually great, often hiding any differences of rate that might exist.

The beginning rate of decay is high. Nitrogen and phosphorus contents of litter determine the length of time that the peak rate will be sustained (Daubenmire and Prusso 1963, Melin 1930, Waksman and Starkey 1924, Witkamp 1971). The decomposition process ties up nitrogen and phosphorus in microbial tissue, while releasing carbon, hydrogen, and oxygen, mainly as carbon dioxide and water. The result is a lower carbon-nitrogen ratio as decomposition progresses (Witkamp 1971).

Temperature and moisture are the major physical factors; and, of these, temperature is the most important (Daubenmire and Prusso 1963, Garrett and Cox 1973, Nelson and Sollins 1973, Reiners 1964, Sollins et al. 1973, Wiant 1967a, 1967b, Witkamp 1971, 1963). The respiration rate of microorganisms will double for each increase of 10°C in temperature, within limits of greater than 10°C and less than or equal to 60°C (Wiant 1967a). However, the mineralization of organic matter continues under snow (Witkamp 1971).

The effect of temperature can be seen in the change of CO_2 evolution with the seasons. The time of greatest CO_2 evolution is in the months of May, June, and July, with the lowest in January, February, and March (Reiners 1964). Temperature is especially limiting in the winter and spring (Garrett and Cox 1973).

The diurnal cycle of CO_2 evolution follows the diurnal air temperature cycle (Witkamp 1969). This is characterized by a pre-sunrise minimum and an afternoon maximum. The time of day when the sample is taken must be taken into account when comparing treatments. Also, if calculations of absolute amounts of CO_2 evolved is to be determined, a 24-hour period of

measurement must be taken (Witkamp 1969).

Moisture content is of importance second only to temperature (Daubenmire and Prusso 1963, Garrett and Cox 1973, Nelson and Sollins 1973, Reiners 1964, Sollins et al. 1973, Wiant 1967b, Witkamp 1963, 1971). The optimum moisture range (dry weight basis) for decomposition by aerobes is 225 to 450 percent (Daubenmire and Prusso 1963). In terms of percent of soil water capacity the optimum range is 60 to 70 percent (Wiant 1967b). Moisture becomes limiting to decay below plant wilting point (Wiant 1967b). Thus, the effects of moisture are found outside of a range from wilting point to almost soil saturation.

Seasonal effects of moisture are best shown in the late summer. The temperature is optimum, but due to desiccation the amount of CO_2 evolved declines, indicating a decline in decomposition (Reiners 1964).

Temperature and moisture account for 75 to 90 percent of the variance involved in measuring litter decomposition by CO_2 accumulation (Reiners 1964, Witkamp 1971). However, due to the heterogenity of organism distribution and species composition, the amount of CO_2 evolved cannot be predicted with accuracy from temperature and moisture parameters.

Methods of Measuring CO_2 Evolution

There are many different ways to collect and/or measure the amount of CO_2 evolved from forest litter. Most of these ways can be adapted to use "in situ" or "in vitro". The three major ways reviewed are: the infrared gas analyzer method, the conductivity method, and the chemical titration method.

The infrared gas analyzer can be used in the field due to development of a mobile system (Nelson and Sollins 1973, Sollins 1973). A number of collection boxes with holes in one end are placed over the forest floor. A line with a dust filter is connected to the end opposite to the air intake holes. This line runs to the mobile gas analyzer mounted in a two-wheeled trailer with recorder, pump, and calibration tanks. The exact set-up of the apparatus is explained by Edwards et al. (1971).

Root respiration is of such magnitude as to require separating the CO_2 collection device from its effect (Nelson and Sollins 1973, Sollins 1973, Witkamp 1971). A shield, made of a gas impermeable material, is placed between the litter horizons and the mineral horizons to seal CO_2 from root respiration from the collection system. A plexi-glass plate was found to best serve the purpose. It is positioned by gently sliding the shield into the horizon from one end (Nelson and Sollins 1973, Sollins 1973). A stainless steel shield was tried first, but it was found to cause a change in the temperature extremes of the collection chamber (Nelson and Sollins 1973).

Litter temperature, relative humidity, and air temperature are determined by thermocouples and a hygrothermograph and recorded on a multipoint recorder also mounted on the two wheel trailer (Nelson and Sollins 1973, Sollins 1973).

There are two main means of collection of CO_2 for the conductivity and the titration methods: the static system and the CO_2 train. Both systems use an alkali solution to collect evolved CO_2.

The static system is composed of a closed container and is most often used in studies conducted in the laboratory (Elkan and Moore 1962, Witkamp

(1966). It is a glass container into which a soil or litter sample and a small container of alkali are placed. The system is then sealed and incubated for the desired time.

The CO_2 train is a system of continual aeration (Waksman and Starkey 1924, Ivarson and Sowden 1959). The air is first passed over soda lime or through an alkali solution which removes CO_2 from it. Next, it is passed through a sulfuric acid solution which removes the ammonia and other short chain volatiles. Then the air is drawn over the litter sample. Finally, the air is bubbled through another alkali solution that absorbs the CO_2 evolved from decomposition of the litter (Black et al. 1965, Ivarson and Sowden 1959, Waksman and Starkey 1924).

The field version of this system is in common use (Humfeld 1930, Marsh 1928, Wallis and Wilde 1957, Witkamp and Van der Drift 1961). The apparatus requires a collector (box, or funnel) inverted over the litter with its edges pushed into the litter and mineral soil to seal it. A tube is used to connect the collector to the alkali container where the collected gas is bubbled through the alkali. A vacuum is held on the alkali container via a tube passing through a safety jar to a vacuum pump. Gas flow is monitored with a flow meter in the vacuum line (Wallis and Wilde 1957, Witkamp and Van der Drift 1961).

The CO_2 collection medium for the conductivity and titration methods is an alkali solution. The general equation, using NaOH as the alkali source is:

$$2NaOH + CO_2 \rightarrow 2Na^+ + CO_3^{2-} + HOH$$

In the system there is an excess of hydroxl ions and the initial concentration of alkali is known. After the alkali is sealed and removed from

the collection system an excess known amount of $BaCl_2$ solution is added to the exposed alkali solution. Following the equation:

$$BaCl_2 + CO_3^{2-} \quad BaCO_3\downarrow + 2Cl^-$$

The carbonate is precipitated out as barium carbonate.

This leaves only the hydroxl alkalinity active in the collection solution. Back titrating to the phenolphthalein end point with a known normal solution of acid gives the amount of alkali not neutralized by CO_2. Thus, knowing the initial and final hydroxl alkalinity, the amount of CO_2 absorbed can be calculated using the formula:

$$CO_2(mgs.) = (B-V)NE$$

B = volume in milliliters of acid to titrate the alkali in the CO_2 collectors from control to end point.

V = volume in milliliters of acid to titrate the alkali in the CO_2 collectors from treatment to end point.

N = normality of acid.

E = equivalent weight, carbon E = 6, carbon dioxide E = 22.

(Black et al. 1965)

The conductivity method is similar to the titration method in its collection of CO_2 by chemical means. It also can be adapted to a continuous read-out system (Newton 1935, Wolf et al. 1952).

The apparatus is made up of a closed container with an alkali solution in it. Fixed to the inside of the cell, suspended in the solution are electrodes. As air from the sample containing CO_2 produced by the decay process bubbles through the solution, the electrical conductivity changes as the CO_2 is absorbed.

This method has evolved from the use of conductivity bridge using a cell with barium hydroxide as the alkali (Newton 1935). This early version has two drawbacks. First, barium carbonate is hard to wash from glassware and secondly, barium carbonate tends to collect on the electrodes and change the value of the cell (Wollum and Gomez 1970).

The drawbacks can be cured by using sodium hydroxide in place of barium hydroxide (Wollum and Gomez 1970). A standardization curve is established by creating various solutions of different concentrations of sodium hydroxide and sodium carbonate and reading their conductivity. From this curve the amount of CO_2 absorbed by a solution can be read directly after a conductivity measurement has been taken.

The advantages of the conductivity method over the titration method are that it is rapid and equipment is readily available (Wollum and Gomez 1970). The absorbing solution is in contact with the atmosphere only a short time. The titration method requires that the absorbing solution be exposed to atmospheric CO_2 while it is being titrated. Thus, the conductivity method has less chance of error from atmospheric CO_2. The conductivity equipment needed is available in most laboratories, while the purchase of automatic titrators to speed up the titration procedure are expensive (Wollum and Gomez 1970).

The theory behind the use of a conductivity bridge and electrical diagrams of it are available in more technical papers (Newton 1935, Wolf et al. 1952).

Sources of Error

There are various sources of error involved in the measure of CO_2 evolution. The greatest natural sources, assuming temperature and moisture constant, are root respiration and loss of carbon as other carbon compounds (Nelson and Sollins 1973, Reiners 1964). Root respiration causes an overestimate, and loss of carbon as methane, acids, alcohols, and other non-CO_2 compounds cause an underestimate of soil biological activity. They vary independently, thus one does not balance out the effects of the other (Nelson and Sollins 1973, Reiners 1964).

The variation of temperature and moisture due to micro-climate effects are great. Areas subject to more intense sunlight are warmer and drier. Thus, temperature and moisture account for as much as 90 percent of the variation involved in measurements of litter decomposition by CO_2 evolution (Reiners 1964, Witkamp 1971).

All of the methods for quantifying CO_2 evolution subject the litter sample to unnatural environmental effects. The infrared gas analyzer method requires a continuous stream of air at a constant rate over the litter sample, as does the carbon dioxide train and conductivity methods. The static method results in formation of a higher than normal CO_2 content and lower than normal O_2 content of the air over the sample. All of these can cause error in the estimates of evolution rate.

There is some question of the validity of using aeration or suction in the collection system because it may introduce error (Brown 1934, Parr and Reuszer 1959, 1962, Smith and Brown 1932, Reiners 1964). Free diffusion (a function of gradients) is the major means of release of CO_2 from the forest floor (Smith and Brown 1932). Various investigators have docu-

mented that as the rate of flow of CO_2 free air increased over the sample, so did the amount of CO_2 evolved from it (Brown 1934, Parr and Reuszer 1962). The same relationship holds for suction.

The static method records approximately 60 percent of the CO_2 recorded by the infrared gas analyzer and carbon dioxide train methods for similar samples and conditions (Nelson and Sollins 1973). The error involved could be due to failure of the alkali to absorb all of the CO_2 and/or a change in the rate of CO_2 evolved. When the air over the litter contains large amounts of CO_2 the growth of anaerobic organisms is stimulated (Parr and Reuszer 1962). The result is a decrease in the amount of CO_2 evolved (Smith and Brown 1932).

When the values obtained by the infrared gas analyzer were compared with the carbon dioxide train it was found the carbon dioxide train recorded 95 percent of the amount of CO_2 evolved as did the gas analyzer (Ivarson and Sowden 1959, Nelson and Sollins 1973). As already stated, the static method recorded only 60 percent. However, earlier workers claim incubation in a closed system is more accurate than is a device using aeration or suction (Smith and Brown 1932).

The titration method of determining the amount of CO_2 absorbed by the alkali solution is subject to other errors in addition to the error present in the collection methods (static or CO_2 train). The errors present are common to any titration: size of drops, overshooting and point, and detecting color change. All are subjective and depend on the expertise of the operator.

The conductivity method is as accurate in determining the amount of CO_2 absorbed in the alkali solution as the titration method (Wollum and Gomez 1970). It is subject to the same collection errors as the titration

method, but in determining the amount of CO_2 absorbed it has different sources of error. The main source of error is that temperature affects conductivity values. The error can be corrected by recording the temperature and applying an adjustment factor or using a temperature compensating device on the conductivity bridge (Wollum and Gomez 1970).

MATERIALS AND METHODS

Area

The study area is located in the northwestern corner of Itasca State Park, Minnesota. It is on the eastern side of Squaw Lake, NE¼, SE¼, Section 5, T143N, R36W, 4th Principal Meridian. The topography of the study area is rolling, with parent material of glacial till. The vegetation is an even-age 55-year-old aspen forest, part of which was clearcut in April 1974. The cut and uncut areas serve as treatments.

Apparatus

The collection system is of the static type, as described in the literature review. It basically is a large vessel enclosing a smaller container with the litter sample and a vial of alkali within. See photo 1.

The main container is an 89 by 170 millimeter labeled glass jar with a black plastic lid and paper seal. Drilled into the lid are two holes, 0.32 cm. and 1.27 cm. diameter respectively. To the 0.32 cm. lid hole a nylon three-way stopcock is attached. The 1.27 cm. hole is sealed with a beveled cork.

The stopcock was to be used in flushing the system with oxygen and to change the alkali solution. It worked for the flushing procedure, but not to change the alkali. The solution was to be changed by inserting a hypodermic needle on a syringe through the stopcock. This approach failed due to cost, scarcity, and tendency of the needles to clog.

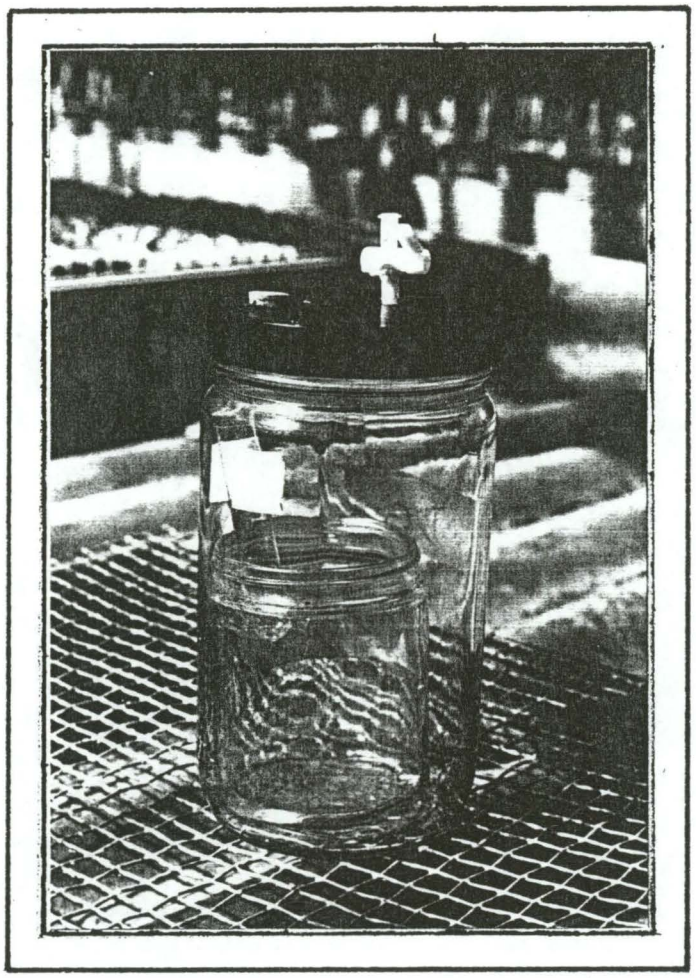

Photo 1. CO_2 collection apparatus

The needles required (9 cm. long) were expensive ($8.00 per dozen) and hard to find. Many needles were needed because they clogged and were impossible to clear.

The 1.27 cm. hole is used as entrance for the oxygen flushing tube. The tube is inserted to the bottom of the container, the stopcock opened, and the oxygen turned on. After approximately 45 seconds the stopcock is shut off and the tube is removed while the oxygen remains on. The oxygen is left on in order to negate any vacuum effects the removal of the tube might create. This partial vacuum could draw outside air into the system, which would result in an error in amount of carbon dioxide recorded.

The sample container within the large outside container is a 55 by 80 millimeter glass jar. Taped to the inside of the large container, above the sample container, is a 6 milliliter plexiglass vial with 5 milliliters of alkali solution in it.

The system described is inexpensive, about $0.92 per apparatus, and is as sensitive as more expensive systems for comparisons between treatments. It is not as accurate as other methods for determination of absolute amounts (Invarson and Sowden 1959).

Field Sampling Procedure

The samples were located randomly. For each month a two-acre portion of each treatment area was chosen using random numbers. Within the section the samples for that month were taken. The individual samples were chosen by throwing a plastic bottle over one's back. The sample was taken where the bottle's cap end landed.

A sample was taken by forcing a bulk density sampler into the litter.

The bulk density sampler used takes a sample 5 centimeters in diameter. The sampler was then dismantled, the sample removed, and measurements made and recorded. Finally, the sample was placed in the carbon dioxide collection apparatus. All of this was done with as little disturbance as possible to the sample. An attempt was made to exclude mineral soil from the litter sample.

Temperature and litter thickness measurements were taken at the time of sampling. The litter temperature was taken with a 12 inch mercury thermometer at the interface of the O_2 and mineral horizons. Only on the first five samples per treatment per month were temperatures taken. These temperatures were taken in order to determine an incubation temperature.

The litter thickness was taken with the use of a pocket rule. The sample was not disturbed when thickness was measured.

In the laboratory the samples were taken out and weighed, the apparatuses flushed with oxygen and 5 ml. of 1.0-N sodium hydroxide solution added. At this time four blanks, jars without litter samples, were prepared in the same way. Finally, all of the systems were placed in the incubator at the temperature of the uncut area.

Originally two incubators were to be used, allowing part of the samples to be incubated at the uncut temperature and part at the cut area's temperature. Due to an equipment problem all of the samples were incubated at the uncut temperature.

After 15 days the alkali solution was removed and labeled. The systems were flushed with oxygen and fresh alkali was added again. They were then placed back in the incubator for an additional 15 days.

Oxygen, although a stimulant to decomposition, was used to flush the systems of atmospheric air. Besides being readily available, commercially bottled oxygen is free from impurities which may cause more serious error. Such impurities include ammonia, carbon dioxide, and water vapor.

The length of time the samples were incubated should have been long enough to minimize the positive effects a pure oxygen atmosphere has on decomposition rate. Fifteen days between replacement of alkali solutions should not have been long enough to cause an oxygen deficiency and subsequent change of aerobic decomposition to anaerobic condition. These conclusions were arrived at subjectively by reviewing other CO_2 evolution studies.

After the second 15 days the alkali was removed and labeled. The litter samples were removed and placed in a drying oven for 24 hours at 80 degrees Celsius. Afterwards, the dry weights were recorded.

The alkali samples were then titrated. The procedure consists of addition of 5 drops of 50 percent barium chloride solution to the 5 milliliter alkali sample. Next it was backtitrated with 0.22-N HCl to the phenolpthalien end point. The amount of acid needed was then used to calculate the amount of carbon dioxide gas evolved per day.

Experimental Design

The experimental design is a completely randomized factorial (Cochran and Cox 1957, Snedecor and Cochran 1967). The mathematical model is:

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha B)_{ij} + \varepsilon_{ijk}$$

where:

$i = 1, 2, 3.$

$j = 1, 2.$

$k = 1, 2, 3, \ldots, 15.$

X_{ijk} = the K^{th} observation in the i^{th} month of the j^{th} treatment.

μ = the true population mean.

α_i = effect of the i^{th} month.

β_j = effect of the j^{th} treatment.

$(\alpha B)_{ij}$ = effect of the interaction of the i^{th} month and the j^{th} treatment.

ε_{ijk} = the error factor for the i^{th} month, j^{th} treatment, k^{th} observation.

The program FACEXP, part of the ISIS statistical package on the University of Minnesota Computer Center's MIRJE system was used to analyze the data. This program uses unweighted cell means in its analysis. In addition, the comparisons between months were tested using Duncan's Multiple Range Test (Steel and Torrie 1960).

A multiple regression was attempted on the data. The general mathematical models for the untransformed data are:

$$CO_2(\text{mgs.})/\text{day} = \alpha + \beta_1 T + \beta_2 M + \beta_3 Th$$

where:

$\alpha, \beta_1, \beta_2, \beta_3$ = constants

T = temperature (°C)

M = percent moisture (dry weight basis)

Th = thickness (cm.)

and

$$CO_2(\text{mgs.})/\text{day} = \alpha\, T\beta M\alpha e^{-\Omega(Th)}$$

α, β, α, Ω = constants

T = temperature (°C)

M = percent moisture (dry weight basis)

Th = thickness (cm.)

e = base of natural logrithm

The program REGANA, also a part of the ISIS statistical program package was used to analyze the data for these models. The step-up method of testing was used to determine the significance of each independent variable (Snedecor and Cocharan 1967).

Each 15-day period per cell was tested for normality with the use of the Kolmogorov-Smirnov test (Lindgren 1968). A t-test of significance was used to compare the amounts of CO_2 evolved for the first 15 days with that from the second 15 days for each cell of months July and August (Snedecor and Cochran 1967). The test consists of taking the difference between the paired readings and calculating the "t" statistic. This statistic is then compared with the value found in a table of Fischer's "t" values with the correct degrees of freedom and desired significance level.

RESULTS AND DISCUSSION

Tables 1 through 6 show at a glance that as the summer progresses differences in temperature and moisture content between treatment areas increase. The cut treatment gets warmer and drier more quickly than the uncut treatment. There is a greater variance in the temperature and moisture contents of the cut area. This is the result of the removal of the overstory which increases the microclimate effect on those parameters.

The litter of the area is generally of the same thickness and composition supporting the assumption that the areas were homogenous prior to treatment. If the areas had not been alike, litter thicknesses and type of litter would have shown a greater variance. The litter was composed primary of aspen leaves and twigs.

The high variance of both temperature and percent moisture, which cause a high variance in CO_2 evolution, could be due to microclimate and microtopography of the area. Even though all samples were taken the same time of day (10:00), those taken on a southern and southwestern aspect were warmer and drier than the northern aspects. This leads to the conclusion that a smaller variance could be achieved if an area with a level topography was used for study.

The June-cut treatment had a higher variation in evolution compared to the other month-treatments. The effect of microclimate, intensified by spring conditions, or of atmosphere leaks in the apparatus are possible causes. Leaks can be ruled out, because if leaks were responsible the

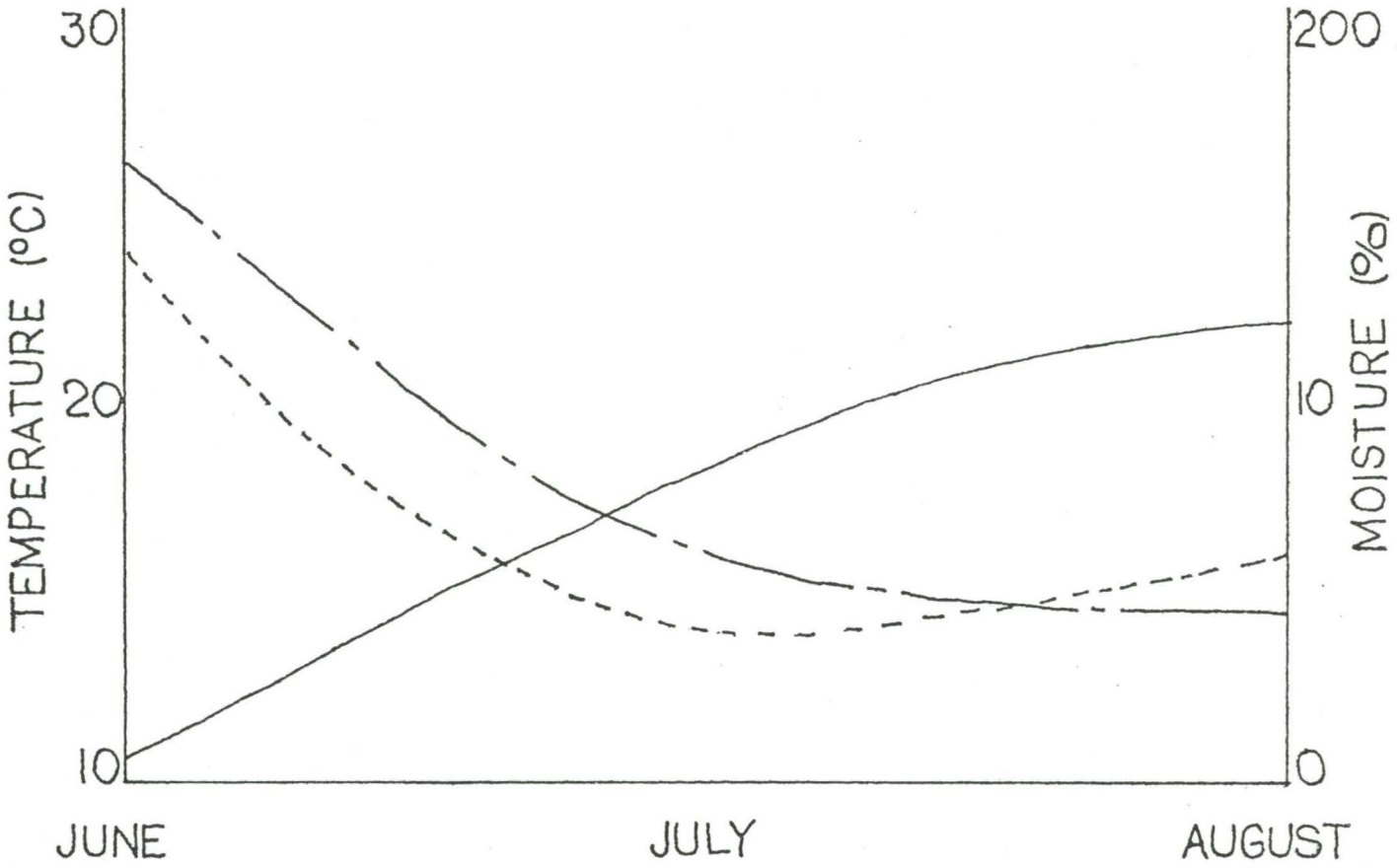

Figure 1. Differences in incubated temperature (solid line), and moisture (cut; dashed line, uncut; broken line).

TABLE 1

Cut - June 19*

sample no.	temperature °C	moisture %	thickness cm.	CO_2/day mg.
1	11.5	144	2.5	1.01
2	8.5	202	2.8	0.62
3	13.5	229	1.6	0.00
4	11.8	106	2.0	3.04
5	13.0	140	2.0	
6	13.8	107	1.8	4.67
7	----	248	2.0	0.49
8	13.2	98	2.0	0.24
9	----	102	1.8	0.85
10	----	135	2.2	0.30
11	----	157	2.2	----
12	----	122	1.6	----
13	----	135	1.8	----
14	----	135	2.0	0.66
15	----	30	2.8	0.91
Averages	12.2	139	2.1	1.16

* incubated at 10.5°C

TABLE 2

Uncut - June 19*

sample no.	temperature °C	moisture %	thickness cm.	CO_2/day mg.
1	10.5	122	2.0	1.20
2	10.0	155	2.0	1.27
3	12.0	94	1.8	1.20
4	11.5	61	1.2	1.04
5	10.5	178	1.5	1.43
6	----	183	1.6	1.14
7	----	298	2.5	1.33
8	----	183	1.8	1.04
9	----	336	2.0	1.04
10	----	130	2.1	1.30
11	----	124	2.2	0.46
12	----	144	2.1	1.30
13	----	128	2.0	0.43
14	----	138	2.1	0.43
15	----	124	1.4	1.08
Averages	10.9	160	1.9	1.01

* incubated at 10.5°C

TABLE 3

Cut - July 1*

sample no.	temperature °C	moisture %	thickness cm.	CO_2/day mg.	
1	22.0	13	3.0	1.22	0.66
2	22.1	42	2.6	3.45	3.60
3	----	54	3.0	3.29	4.18
4	20.0	79	3.0	2.58	3.63
5	----	21	2.0	0.70	0.91
6	----	96	3.0	1.57	2.04
7	----	20	2.6	2.09	1.82
8	----	22	2.5	2.41	2.85
9	----	40	4.0	2.93	3.66
10	----	66	2.5	1.67	1.43
11	----	17	1.9	0.96	0.65
12	----	47	3.2	2.45	2.72
13	----	19	1.8	1.25	1.08
14	----	27	2.0	1.35	1.40
15	----	44	4.0	3.06	3.60
Averages	21.4	40	2.7	2.06	2.28

* incubated at 18°C

TABLE 4

Uncut - July 1*

sample no.	temperature °C	moisture %	thickness cm.	CO_2/day mg.	
1	18.1	81	2.5	2.14	3.01
2	18.0	60	3.0	2.12	3.07
3	18.0	54	3.5	3.00	3.89
4	17.0	70	2.0	3.32	3.60
5	----	60	4.0	3.67	2.14
6	----	66	3.5	2.80	3.60
7	----	64	2.0	1.86	2.50
8	----	49	3.0	2.64	3.27
9	----	55	2.0	1.54	1.53
10	----	48	2.3	2.60	3.47
11	----	70	2.5	2.61	3.27
12	----	52	4.0	3.80	4.40
13	----	46	3.0	2.06	3.69
14	----	69	4.0	3.24	3.40
15	----	58	2.2	1.54	2.21
Averages	17.8	60	2.9	2.60	3.14

* incubated at 18°C

TABLE 5

Cut - August 4*

sample no.	temperature °C	moisture %	thickness cm.	CO_2/day mg.	
1	23.0	67	2.5	2.48	3.92
2	22.0	99	3.0	2.59	4.20
3	22.0	54	2.5	2.18	3.73
4	21.0	7	1.0	0.00	1.79
5	24.5	68	3.5	2.20	4.35
6	----	67	2.0	2.38	3.84
7	----	91	4.5	2.49	4.00
8	----	62	3.4	2.49	5.48
9	----	77	3.2	2.45	4.06
10	----	70	1.8	2.44	3.19
11	----	42	2.0	2.37	3.64
12	----	62	3.0	1.61	2.23
13	----	59	2.4	2.20	3.55
14	----	10	0.5	----	2.19
15	----	28	3.0	2.57	3.73
Averages	22.5	58	2.6	2.18	3.59

* incubated at 22°C

TABLE 6

Uncut - August 4*

sample no.	temperature °C	moisture %	thickness cm.	CO_2/day mg.	
1	21.5	57	2.5	2.32	3.44
2	22.0	48	2.5	2.65	2.70
3	22.0	38	4.0	2.23	3.73
4	21.0	47	1.5	2.14	3.07
5	----	61	4.0	2.18	2.67
6	----	47	2.5	2.39	4.12
7	----	45	2.8	2.39	3.62
8	----	51	2.0	2.46	3.48
9	----	62	3.5	2.95	0.05
10	----	49	2.3	2.57	4.24
11	----	23	2.5	2.06	3.95
12	----	42	3.8	3.20	4.28
13	----	31	1.6	2.61	3.40
14	----	43	3.0	2.31	3.35
15	----	75	3.5	3.22	----
Averages	21.6	48	2.8	2.51	3.29

* incubated at 22°C

TABLE 7

Factorial ANOVA

Source	D. F.	Means Sqs.	F-Statistic	Significance ($p \leq 0.05$)
Month	2	36.002	36.613	Yes
Treatment	1	1.164	1.164	No
Month x Treatment	2	1.915	2.107	No
Error	138	0.909		

The statistical analysis does show a significant difference between months (see table 7). This difference is due to an increase in CO_2 evolved with each month. A comparison using Duncan's Multiple Range Test shows that the differences between June and August, and between June and July are significant ($p = 0.05$), with no difference shown between July and August.

The results of the comparisons show the limiting effects of temperature and moisture. The June to July increase in CO_2 evolved demonstrates the effect an increase in temperature can have. A greater temperature increases the activity of the microorganisms and thus, increases the rate of CO_2 evolved.

The nonsignificant difference between July and August illustrates the effect of low available moisture. In tables 3, 4, 5, and 6 the average litter temperature increase of 4°C has a nonsignificant effect on the CO_2 evolved. However, the average moisture content of the treatments remains about the same. This would seem to indicate that the moisture content

arrived at in July and sustained in August is critical to further activity by the microorganisms (see tables 3, 4, 5, and 6).

The two 15-day incubation periods for both treatments in July and August are significantly different ($p = 0.05$). The test used to compare the two periods for each treatment and month is a t-test of paired data (Snedecor and Cochran 1967). The results show a significant difference between each first 15-day period and the second. These differences are thought to be due to a change in moisture content between periods. Moisture differences are due to the use of water in decomposition and loss of moisture with flushing of the system prior to installation of the second alkali sample.

REGRESSIONAL MODEL(s)

As explained earlier, temperature, moisture content, and total litter thickness measurements were recorded for each sample. The purpose, with the use of multivariate regression analysis was to attempt to predict the milligrams of CO_2 evolved per day per sample. If such a relationship of an accurate enough nature could be found, the amount of CO_2 evolved per area per unit time could be quantified by simpler and quicker measurements of the related parameter(s).

The linear regression model does not fit the data (see table 8). Of the three parameters used to predict CO_2 evolution only temperature was significant (see table 8). The correlation coefficient, 0.483, though significant, is poor.

TABLE 8

Linear Regression ANOVA

Source	D. F.	Mean Sqs.	Indiv. "R"
Temperature	1	11.805	+0.483
Added by Moist.	1	1.026	-0.275
Added by Thick.	1	0.0469	+0.279
Lack of Fit	23	1.222	
Pure Error	14	0.683	

TABLE 9

Calculated F-Statistics

Step Up Method of Regression Model Analysis

Statistical Hypothesis	Linear Model	Curvilinear Model
H : $\beta_{temp.} = 0$	11.87* significant	16.31* significant
H : $\beta_{moist.} = 0$	3.20* nonsignificant	0.002* nonsignificant
H : $\beta_{thick.} = 0$	3.30* nonsignificant	2.69* nonsignificant
H : Adequacy of Fit	1.79* nonsignificant	4.09** significant

* F table value, p = 0.05, with 1, 39 degrees of freedom = 4.08

** F table value, p = 0.05, with 15, 22 degrees of freedom = 2.31

The curvilinear model is significant for the test of adequacy of fit (see table 9). In this model too, only temperature proved to be significant ($p = 0.05$) in the step-up method of analysis of independent regression factors (see table 9). Therefore, the best prediction model for these data is:

$$C = 1.16 - 2.552 \ln T$$

where:

C = milligrams of CO_2 evolved per sample per day.

T = litter temperature in degrees Celsius.

The correlation coefficient of temperature for both models would be higher if each sample had been incubated at the temperature at which it was collected. Even though incubated at a different temperature, the temperature of the litter sample when collected correlates well due to its continued effect on microorganism population numbers and species distribution. These effects are: greater population with higher temperature and a bias towards more active aggressive species with higher temperature.

Moisture would be expected to show a significant correlation with the amount of CO_2 evolved per day due to its direct effect on microorganisms as discussed in the literature review. A reason for the lack of correlation could be due to the two 15-day periods of determination of CO_2 evolution for each litter sample. The moisture contents between the two periods may have crossed a value critical to CO_2 evolution. Either side of this critical moisture value the relationship of CO_2 rate change to change in percent moisture is significantly different. This would not allow the use of the calculated moisture content based on the weight of the sample before the first 15-day period to be used for the second 15-day period also. Since

it is possible to get only one oven-dry weight for each litter sample, litter samples should be used for only one carbon dioxide run.

The thickness of the litter horizon is not statistically correlated with the amount of CO_2 evolved per day. However, the amount of CO_2 evolved per day would, over a period of time, possibly years, determine the litter thickness. In this study, litter thickness is not sensitive enough to small changes in CO_2 evolution rate to correlate with it.

Apparatus and Procedural Error

It was found that the simplest apparatus design was the best. A jar without holes in the lid is the best. The flushing of the system with oxygen can be discontinued as long as blanks are used to estimate atmospheric system noise. As a result the holes in the top and their possible introduced error can be emitted.

In the titration procedure a possible error caused by addition of barium chloride was made. It is questionable if an excess of barium chloride was added to each alkali sample. If not, there is error introduced by the tying up of hydrogen ions from the acid by the few carbonate ions being changed to bicarbonate ions. This error is probably greatest in the higher evolution rates due to greater amounts of carbonate and bicarbonate ions from the large amount of CO_2 absorbed. Nothing can be done to calculate this error.

SUMMARY

This experiment found no difference in CO_2 evolution rates from the forest floor of the cut and uncut treatment areas. This was caused by either microclimate and topographic effects, or by procedural error.

A difference was shown between months, caused by differences in litter temperature. But very little, if any moisture effects could be shown. This was possibly caused by the moisture content of the litter not falling below the soil wilting point.

It was also found that a simple apparatus design is the best when using chemical means of quantifying CO_2 evolution. Blanks should be incubated along with the samples to quantify atmospheric noise in the system.

APPENDIX

TABLE 10

	July		August	
	Cut	Uncut	Cut	Uncut
Differences	+0.56	+1.53	-1.44	-0.49
	-0.15	-0.80	-1.61	-1.12
	-0.89	-0.64	-1.46	-1.73
	-1.05	-0.63	-1.51	-1.23
	-0.21	+0.01	-2.99	-1.02
	-0.47	-0.87	-1.61	-1.67
	+0.27	-0.87	-1.55	+2.90
	-0.44	-0.66	-0.75	-1.89
	-0.73	-0.60	-1.27	-1.08
	+0.24	-0.95	-0.62	-0.05
	+0.31	-0.89	-1.35	-0.79
	-0.27	-0.28	-1.79	-1.50
	+0.17	-1.63	-1.16	-0.93
	-0.05	-0.16	-2.15	-1.04
	-0.54	-0.67	----	----
Mean Difference	-0.217	-0.541	-1.417	-0.765
Standard Error	0.112	0.184	0.153	0.328
Calculated "t"	1.77	2.94	9.24	2.33
Table "t" (p=0.05)	2.14	2.14	2.16	2.16
Significance	no	yes	yes	yes

TABLE 11

Data Summary Table

	Treatment	June	July		August	
	Mean mg. CO_2/day =	1.16	2.06	2.28	2.18	3.59
(CUT)	Variance =	1.41	0.89	1.24	0.67	0.94
	Coef. of variation	1.22	0.43	0.55	0.31	0.26
	Mean mg. CO_2/day =	1.01	2.60	3.14	2.51	3.29
(UNCUT)	Variance =	0.30	0.72	0.76	0.36	1.06
	Coef. of variation	0.28	0.28	0.24	0.14	0.32

TABLE 12

Curvilinear Regression ANOVA

Source	D.F.	Means Sqs.	Indiv. "R"
Temperature	1	4.523	+0.543
Added by Moist.	1	1.746	-0.071
Added by Thick.	1	0.090	+0.254
Lack of Fit	22	0.350	
Pure Error	14	0.086	

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